Reduction of Mutagen Formation in Cooked Nitrite-Free Bacon by Selected Cooking Treatments

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ABSTRACT

A newly detected cooking-generated promutagenic fraction in nitrite-free bacon was evaluated to determine the heating conditions required for its formation. This apparent nonnitrosamine mutagenic activity was detected by the Ames mutagenicity assay when the product was heated by frying, baking, broiling, or high temperature autoclaving, but was not detected in microwave irradiated, steamed, or conventionally autoclaved samples. The ingestion of the genotoxin(s) can be avoided by employing low temperature cooking methods or strictly controlling heating time. The toxicologic significance of exposures to this genotoxic fraction is unknown.

INTRODUCTION

THE DEVELOPMENT of rapid in vitro bioassays has allowed investigators to detect mutagens in a number of heat-processed foods. These include: beef, chicken, potatoes, evaporated milk, bakery and pasta products, breakfast cereals, fish, and pork products (Spingarn et al., 1980; Felton et al., 1981; Levin et al., 1981; Krone and Iwaoka, 1981; Pariza et al., 1979a; Miller and Buchanan, 1983). Observations from model systems and heat-processed foods suggest that these mutagens may be the result of Maillard browning reactions, although, in most cases the chemical identities have not been determined. Environmental mutagen exposure is of concern because their low chronic level presence may contribute to the etiology of human neoplasms by carcinogenic initiation, an increase in the mutational load on germ cells, or congenital malformations (Brusick, 1980). The public health significance of heat processing generated mutagens is unknown.

Recently, we reported the detection of a promutagenic fraction in fried nitrite-treated and nitrite-free bacon using the *Salmonella* mammalian/microsome mutagenicity assay (Miller and Buchanan, 1983a). Activity was detected with frameshift tester strains TA 98 or TA 1538, and had a strict requirement for metabolic activation. A dose-response relationship was observed also in fried green pork belly slices, indicating that the precursors are endogenous to the tissue and therefore not introduced during the curing, smoking, or initial heat processing stages of bacon production. Chemical analyses indicated that the mutagenicity was not due to a volatile nitrosamine or benzo(a)pyrene (BaP). Added proof that the activity is not from a nitrosamine comes from our observation that the bacon genotoxicity is not responsive to TA 100 which is used to detect base-pair substitution mutations. Preliminary kinetic experiments showed that the mutagen was formed in conjunction with frying temperatures above 125°C.

Spingarn and Weisburger (1979) demonstrated the presence of mutagens in broiled and fried beef. Surface temperatures were 125°C and 130°C for fried and broiled product, respectively. Dolara et al. (1979) predicted and observed a sharp increase in mutagen formation in hamburgers when heated to 140–180°C. This group observed no mutagen production in microwave-heated hamburgers. Pariza et al. (1979b) tested fried hamburgers and observed high levels of mutagens when product was fried for 10 min at 190–210°C. Nader et al. (1981) observed no mutagenic activity in microwave heated beef.

Since previous studies have indicated that mutagen formation in heated meat is time/temperature dependent, this suggested that the level of the bacon mutagen(s) could be reduced or eliminated by selected cooking parameters that minimize mutagen formation. Therefore, the objective of this study was to determine how various cooking methods influence formation of genotoxins in nitrite-free bacon.

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MATERIALS & METHODS

NITRITE-FREE BACON was prepared by pumping 4–5 kg pork bellies to a weight 10% above green weight using a Koch stitch pump injector. Curing solution consisted of 770g distilled/deionized water, 30g sodium tripolyphosphate, 50g sucrose, and 150g food grade sodium chloride per liter. Bellies were cured overnight at 0.5°C, and then processed by a 5-hr cooking/smoking schedule to an internal temperature of 53°C. Smoke was generated with hardwood chips and applied for the final 3-hr period. The finished product was stored overnight at 0.5°C, tempered the following day to approximately −4°C, sliced into 3-mm strips, and then wrapped in aluminum foil and stored at 4°C. Prior to final cooking treatments, the 100g bacon samples were equilibrated to room temperature.

Heating methods

Electric skillet frying. Nitrite-free bacon strips were fried for 4–7 min in a preheated Teflon-coated electric skillet (Presto, Model PA07A) calibrated to 171°C (range 150–199°C). Strips were turned once during the procedure. Grease and char were removed from the pan between batches.

Broiling. A gas oven/broiler (Tappan E0K1V-31) was preheated to 250°C (range 180–316°C). Product was placed on a warm broiling pan and broiled for 0–5 min, 15 cm from the flame.

Baking. The gas oven was preheated to 177°C (range 145–218°C) and bacon strips were baked on a warm broiling pan for 0–25 min in the center of the oven.

Autoclaving. Bacon strips were heated for 0–60 min at 15 psi (121°C) on a broiling pan. In further experiments, bacon was heated at 75 psi (160°C) for 0–60 min. This 75 psi treatment yielded bacon that was considered unedible.

Steaming. Bacon strips were steamed for 0–12 min on a screen over boiling water.

Microwave irradiation. Product was cooked for 0–3 min in a Litton (model 550) microwave oven (1200W, 2450 MHz). Beyond 3 min the product was not considered edible.

Except for the microwave oven and high pressure autoclave treatment, internal product and atmospheric temperatures were monitored by an electronic temperature recorder (Bailey Instruments, Inc., Model BAT-B) equipped with copper-constantan electrodes. All processes were performed on a minimum of two bacon samples. Except as noted above we considered all product edible. Heated samples were stored at −18°C until extracted, always within 48 hr. 100g samples of nitrite-free bacon were used for all experiments.

Mutagen extraction. The mutagen extraction scheme was modified from that of Felton et al. (1981). Frozen bacon slices were reduced to a powder by blending with solid CO_2 in a food processor. The powdered sample was transferred to a beaker and homgenized in methanol (5 ml/g) with a Brinkmann polytron homogenizer Model PCU-2. The homogenate was filtered, and the solids were

reextracted and refiltered. Methanol extracts were pooled and stored overnight at −80°C, and the cold extracts were filtered to remove precipitated materials. Solvent was removed by evaporating in vacuo.

The residue was dissolved in 0.01N HCl (pH 2), and extracted three times with 2 ml/g dichloromethane (DCM). This fraction was discarded since preliminary studies have indicated that this fraction exhibited no mutagenic activity. The aqueous portion was adjusted to pH 10 with NaOH and reextracted with DCM. This alkaline/organic extract was pooled and concentrated in vacuo. Extracted samples were stored at −18°C in 1 ml of DCM while awaiting mutagenicity assay. Immediately prior to the assay, a portion representing 10% of the total sample was removed, evaporated under nitrogen, redissolved in dimethyl sulfoxide (DMSO), and tested for mutagenic activity.

Mutagenicity assay. The histidine-requiring strain TA 98 of *Salmonella typhimurium* (a gift of Dr. B.N. Ames, Berkeley, CA) was employed as the tester organism. The plate incorporation method described by Ames et al. (1975) was employed with minor modifications to detect mutagen-induced His^+ revertants. Minimal medium consisted of 400 mM K_2HPO_4, 150 mM $MgSO_4$, 15 mM sodium citrate, 75 mM $(NH_4)_2SO_4$, 1.5% agar, and 220 mM glucose. Soft top agar (0.5%) included 0.6% NaCl, and trace amounts of histidine and biotin. Molten (50°C) top agar was distributed in 2-ml portions into tubes, and then supplemented with 10^8 cells, 0.1 ml DMSO containing controls or test fractions, and 0.5ml of S-9 mix (2.8 mg protein) or phosphate buffer. The S-9 mix was prepared from Aroclor 1254-induced rat livers. The top agar mixtures were overlayed on prepoured minimal medium plates. Plates were scored for histidine prototrophy after incubation at 37°C for 48 hr. BaP (10 μg) and 2-acetylaminofluorene (10 μg) were included as positive controls. Duplicate or triplicate plates were assayed for each sample. A sample was considered positive if it produced a twofold increase above the spontaneous reversion rate, and exhibited a dose-response relationship. Prototrophy on some experimental plates was confirmed by randomly picking 10 colonies, transferring them to histidine-free, biotin-supplemented, minimum medium plates, and incubating for 48 hr at 37°C. Only true His^+ organisms would grow on this medium.

Electric skillet frying

Results obtained from bacon samples fried at 171°C in an electric skillet are shown in Fig. 1. After an initial lag of 5 min, mutagenic response increased with longer frying times to 6.5 min (∿fivefold). Further frying resulted in decreased genotoxicity (threefold above controls), presumably due to violation or degradation of the compound. The internal temperture of the fried bacon samples reached a maximum of 128°C; maximum mutagenic activity was observed concommitantly at this temperature. Subsequent heating yielded fewer revertants, yet internal temperatures remained constant.

Taylor et al. (1982) suggested that mutagen formation may be oxygen dependent in deep fat fried meat products. Therefore, bacon samples were fried at 171°C covered with a lid and flushed with nitrogen gas, covered and purged with air, and uncovered in ambient air. All three samples were heated for 6 min without turning. Increases in mutagenicity were: 2.3, 3.0, and 2.8-fold above control samples for the nitrogen purged, air purged, and ambient air samples, respectively. An analysis of variance demonstrated that these means were not significantly different at the 95% level. These data suggest that genotoxin formation is not oxygen dependent.

Broiling

The effects of broiling on mutagenic activity in nitrite-free bacon are shown in Fig. 2. A 2-min lag in detectable genotoxicity was observed. The 5 min samples yielded high (160-fold) levels of activity. Broiling produced the largest amount of genotoxic activity. Further heating resulted in a decrease in activity in a manner similar to the fried samples. At 6 min there was a twelvefold increase in activity. The

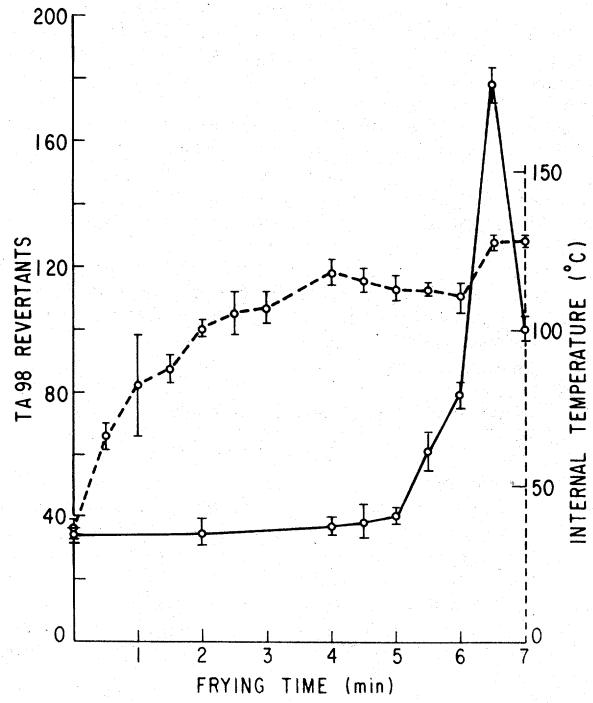

Fig. 1—Frying effects at 171°C on mutagenic activity in nitrite-free bacon (5g-equivalent uncooked bacon). Dotted line indicates mean (±SD) of internal temperature; solid line indicates mean (±SD) of mutagenic response (triplicate plates, in duplicate).

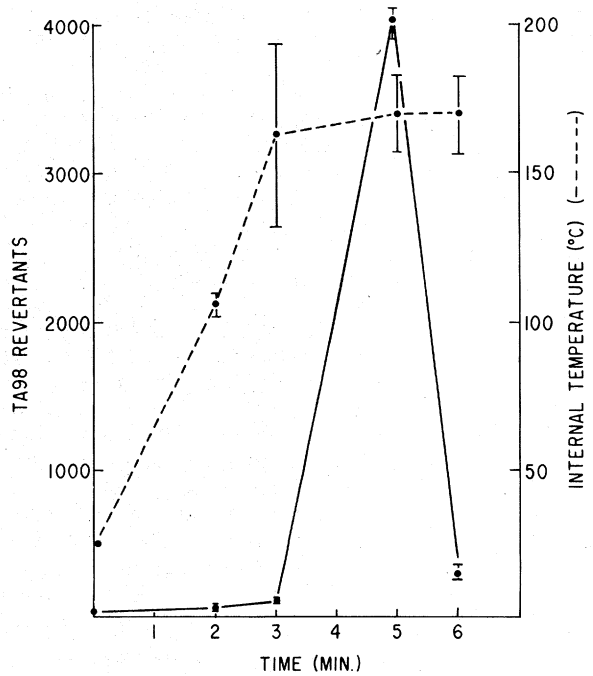

Fig. 2—Broiling effects at 250°C on mutagenic activity in nitrite-free bacon (5g-equivalent uncooked bacon). Dotted line indicates mean (±SD) of internal temperature; solid line indicates mean (±SD) of mutagenic response (triplicate plates, in duplicate).

maximum internal temperature reached in the broiled samples was 170°C, and maximum mutagenic response occurred in conjunction with that temperature.

Baking

Data from bacon baked at 177°C are presented in Fig. 3. Similar to the above heating methods, there was an observable lag period when no mutagenic response could be detected. In the baked samples no genotoxic activity was observed through the 12.5-min sample. An approximately fivefold increase in the number of revertants was then observed in the 17.5-min sample, while the 25-min sample produced an elevenfold mutagenic increase over background levels. Maximum internal temperature was 144°C, found in the 25-min sample.

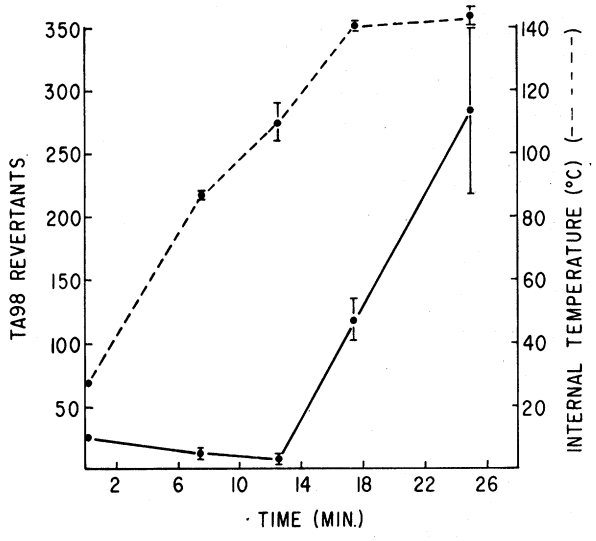

Fig. 3—Baking effects at 177° C on mutagenic activity in nitrite-free bacon (5g-equivalent uncooked bacon). Dotted line indicates mean (±SD) of internal temperature; solid line indicates mean (±SD) of mutagenic response (triplicate plates, in duplicate).

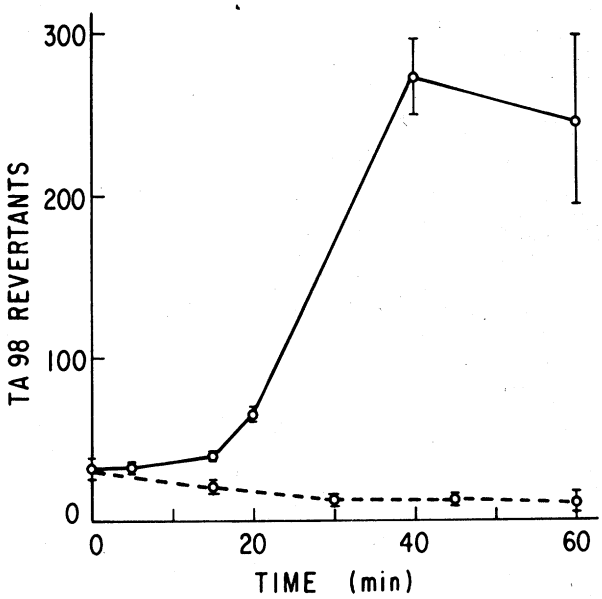

Fig. 4—Autoclaving effects at 121° C (dotted line) and 160° C (solid line) on mutagenic activity in 5g-equivalent nitrite-free bacon (duplicate plates, in duplicate).

Steaming

Steaming bacon did not produce any mutagenic activity over a heating period of up to 12 min.

Microwave irradiation

Bacon that was microwave irradiated for 0–3 min demonstrated no mutagenic activity.

Autoclaving

Bacon strips, autoclaved at 121°C for 0–60 min, contained no detectable mutagenic activity (Fig. 4). Maximum internal temperature of the product was 119°C. Bacon samples were also heated in a high pressure autoclave to 75 psi (160°C) for 0–60 min. These data are included in Fig. 4. After the initial lag an increase in genotoxicity was observed in the 20-min (twofold) and 40-min (eightfold) samples. Heating to 60 min resulted in a slight decrease, but still strongly positive (sevenfold), compared to the 121°C autoclaved samples.

Analysis of mutagenic data

Data from the linear portion of the dose/response curves for the fried, broiled, and baked samples were plotted against the corresponding internal temperatures (Fig. 5). A second order equation was derived from these data and is as follows: $Y = 0.00013X^2 - 0.015X + 1.77$. The data suggested that the differences among the cooking methods that produce the bacon genotoxicity appear to be largely a function of the internal temperature of the bacon strips.

DISCUSSION

THE MUTAGEN-FORMING POTENTIAL of nitrite-free bacon was determined by heating the product by various cooking methods. Results demonstrated that mutagen levels were proportional to internal temperature of the cooking product. Similar findings were reported by Spingarn and Weisburger (1979) and Dolara et al. (1979) when cooked ground beef samples were tested for mutagenic activity. Because of the thickness of the product, genotoxic gradients were observed, with the outer portions containing higher activity than center of the hamburgers.

The present study demonstrated that prolonged exposure to high heat resulted in decreased genotoxicity in bacon as previously reported (Miller and Buchanan, 1983). Others have observed this phenomenon in heated ground beef

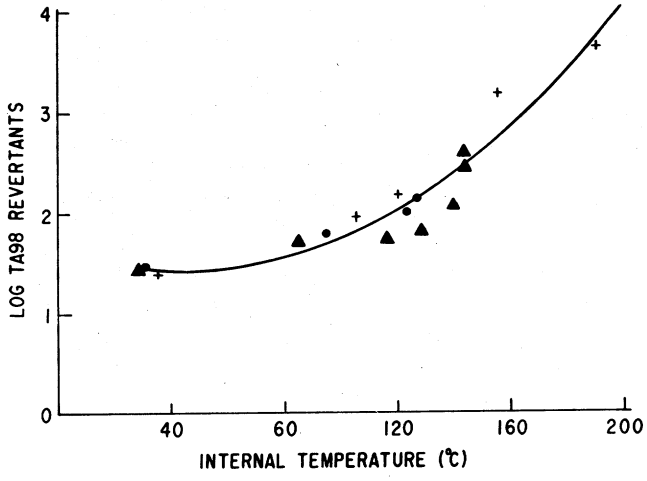

Fig. 5—Heat treatment effects on mutagen formation in nitrite-free bacon (+ broiled, • baked, ▲ fried).

(Bjeldanes et al., 1982). Rappaport et al., (1979) reported that decreased mutagenic levels upon extended heating are attributable to volatilization of the active agent in ground beef. Felton et al. (1981) confirmed this observation.

Lack of mutagen generation by microwave heating is consistant with observations by others who studied microwave heating of beef (Nader et al., 1981). Our exposure times were short as compared with these investigators but, bacon is a thinner product that would absorb heat faster than hamburgers, and the wattage applied in our study (1200 W) was double that used by the Australian group. At 3 min of heating, the product was considered inedible.

The findings of the present study suggest that combinations of low temperature and short cooking durations may be efficacious for decreasing the formation of genotoxins incurred during the cooking of bacon. Low temperature heating methods such as steaming, low temperature autoclaving, and microwave cooking may be effective methods to prepare bacon without a risk of producing mutagens. Likewise, nitrosamine formation can be reduced by lower cooking temperatures and shortened cooking times (Pensabene et al., 1974). However, the utilization of both methods to control mutagen formation must assure destruction of parasites, especially *Trichinella.*

Three N-substituted aromatic amines having genotoxic activity have been isolated and quantified in moderately heated beef and fish: 2-amino-3-methylimidazo [4,5-f] quinoline (IQ), 2-amino-3,4-dimethylimidazo [4,5-f] quinoline (MeIQ), and 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline (MeIQx) (Sugimura, 1982; Hargraves and Pariza, 1983; Yamaizumi et al., 1980). These compounds display genotoxic activity similar to that observed with the mutagenic fraction in the present study. However, at the present time it is not known if IQ, MeIQ, or MeIQx are present in the mutagenic fraction from bacon. Work to confirm their presence or absence is currently in progress.

The health effects of dietary genotoxins remains a valid question without a definitive answer. Disease and food consumption patterns (Doll and Peto, 1981) indicate the association of certain human neoplasms (colon, rectum, breast, prostate) with a number of dietary components (meat, fat, calories, lack of fiber), but the epidemiological results are not consistant. Genotoxins have been observed in a variety of food products. Considerable research has been directed toward products that exhibit both genotoxic activity and show statistical relationships with human cancers. Epidemiologically, nutritional determinants including pork consumption have been implicated as causative factors in neoplasia (Lubin et al., 1981). Bjeldanes et al. (1982) and Miller and Buchanan (1983) reported the detection of genotoxic activity in bacon and other pork products. More significantly, Baker et al. (1982) detected mutagenic activity in human subjects' urine following ingestion of fried pork or bacon. The strain specific and activation characteristics of the mutagen(s) detected in the urine samples were similar to those of the cooking-generated mutagen(s). Baker et al. (1982) concluded that the cooking generated mutagen(s) can be absorbed, and excreted in urine by humans. It would seem prudent therefore, to reduce exposure to these mutagenic compounds. The present study has demonstrated that this can be accomplished by altering traditional cooking methods.

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Precautions should be exercised in the handling of these mutagenic extracts since most mutagens are also carcinogens.

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